Reference: *Biol. Bull* **184**: 125–143. (April, 1993)

Gametogenesis and Spawning of the Sea Cucumber *Psolus fabricii* (Duben and Koren)

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Abstract. The reproductive cycle of the sea cucumber *Psolus fabricii* was studied in a population from the St. Lawrence Estuary in eastern Canada from May 1988 through August 1989. The gonad consists of numerous germinal tubules which vary greatly in size. The mean diameter of the tubules and gonadal mass follow annual cycles, increasing from early winter through spring, and dropping abruptly during spawning in the summer. Gametogenesis is generally a prolonged process and begins in small tubules in January. By summer the ovarian tubules contain oocytes with a modal diameter of 400–600 μm, and the testicular tubules contain an abundance of early spermatogenic stages, but rarely spermatozoa. These small tubules of the gonad do not spawn until the following year, and there is little gametogenic activity within them until January, when oocyte growth and the production of later spermatogenic stages resumes. The latter production continues until summer and results in a marked increase in the diameter of the tubules. Then, during spawning, these now large fecund tubules are transformed into small tubules. Following spawning, the predominant activity within the spent tubules is phagocytosis of the residual gametes. The active phase of gametogenesis (January to summer) coincides with an increasing photoperiod regime, and an accelerated gametogenesis occurs in March when temperature and food availability begin to increase. Spawning was one month later in 1989 than in 1988 and did not show a consistent relationship with either temperature or light conditions. However, in both years, spawning coincided with a decrease in the freshwater run-off into the Estuary and with the predicted annual increase in phytoplankton.

Received 2 April 1992; accepted 25 January 1993.

Introduction

If we want to describe the reproductive cycle of an invertebrate, we need information about the gonad (structure and development) and gametogenesis (with respect to biometry) (Smiley *et al.*, 1991), and about environmental factors that control these events (Giese and Pearse, 1974; Himmelman, 1981). We are interested in reproduction in echinoderms and particularly in holothurians. The gonad of many holothurians is unusual in that it consists of numerous germinal tubules (Théel, 1882; Tyler and Gage, 1983; Smiley and Cloney, 1985; Smiley, 1988) that can vary markedly in size and state of gametogenic development (Théel, 1901; Kille, 1939, 1942; Smiley and Cloney, 1985; Smiley, 1988; Smiley *et al.*, 1991). Smiley (1988) shows that, in female *Stichopus californicus,* fecund tubules are attached to the posterior part of the gonad. He suggests that this arrangement may be a general characteristic of holothurians. Because of the complex gonadal morphology, seasonal changes in gametogenesis are more difficult to quantify in holothurians than in invertebrates that have a globular gonad. Another problem in studying holothurian reproductive cycles is that body size can vary drastically due to water uptake and loss (Edwards, 1910) so that body component indices are not as constant and reliable as they are for many other invertebrates. The principal studies on holothuroid reproduction are those by Tanaka (1958), Krishnaswamy and Krishnan (1967), Rutherford (1973), Green (1978), Engstrom (1980), Conand (1981, 1982), Tyler and Gage (1983), Costelloe (1985), Smiley and Cloney (1985), Cameron and Fankboner (1986), Tyler and Billett (1988), Smiley (1988), Bulteel *et al.* (1992), and Sewell (1992).

The role of environmental factors in controlling gametogenesis and spawning in marine invertebrates has

been well investigated. Temperature, food availability, and photoperiod have often been suggested as environmental cues based on correlative evidence (Giese and Pearse, 1974; Himmelman, 1981; Todd and Doyle, 1981; Giese and Kanatani, 1987). Laboratory experiments in conjunction with field observations have demonstrated that specific environmental changes coordinate certain reproductive events in echinoderms. For example, photoperiod has been shown to be the primary factor controlling gametogenesis in the urchin *Strongylocentrotus purpuratus* and the seastar *Pisaster ochraceus* (Pearse and Eernisse, 1982; Pearse *et al.*, 1986). In addition, spawning in several echinoderms as well as molluscs, seems to be triggered by the spring phytoplankton increase (Himmelman, 1975, 1981; Starr, 1990; Starr *et al.*, 1990, 1992). Temperature (Tanaka, 1958), light intensity (Conand, 1982; Cameron and Fankboner, 1986), water turbulence (Engstrom, 1980), salinity (Krishnaswamy and Krisnan, 1967), a combination of temperature and light intensity (Costelloe, 1985), and phytoplankton blooms (Cameron and Fankboner, 1986) have all been suggested as potential spawning cues particularly for holothurians, but experiments demonstrating such a role have yet to be performed.

In this study we examined the reproductive cycle of the holothuroid *Psolus fabricii* (Duben and Koren) in relation to environmental conditions for a period of 16 months. This species was chosen because it possesses the complex system of gonadal tubules characteristic of holothurians, and because it can be readily collected since it is abundant on rocky faces in the subtidal zone in our region. First, we developed techniques for quantifying changes in the gonads and associated body organs. Because of the marked variation in the size and state of development of the germinal tubules, we decided to quantify the gametogenic events in large and small tubules separately, so as to clarify the extent to which tubule size affects interpretation. Although previous studies on holothurians report differences in gametogenetic development in tubules of different size, this is the first study quantifying the rate of gametogenic development in different-sized male and female tubules at frequent intervals throughout the year.

Materials and Methods

Study site

The population studied was at Anse à Robitaille (48°32′ N; 69°41′ W), 2.5 km from Les Escoumins on the north shore of the lower St. Lawrence Estuary. Samples of 35–40 individuals were collected at monthly or bimonthly intervals between May 1988 and August 1989, from a bedrock face (45°–60°) at a depth of about 10 m below the lowest water of spring tides. Because the animals could not be dissected immediately, they were preserved in 10% neutralized formalin in seawater and dissected about a month later. By this time, changes due to the preservation should have stabilized (Pitmann and Munroe, 1982).

Determination of indices of the gonad, respiratory tree, and intestine (including its contents)

The dry mass of the body wall, including the aquapharyngeal bulb, longitudinal muscle bands, and cloacal muscles (Fig. 1A), was chosen as a denominator for body component indices because calculating these indices as a proportion of the wet body wall mass would have markedly increased the confidence intervals (by 12–25% for the gonadal index, 9–18% for the intestinal index, and 16–22% for the respiratory tree index). Moreover, seasonal variations in dry body wall mass were probably small since the calcareous plates accounted for ≈87% of this mass. All masses were recorded to the nearest 0.01 g, and dry masses were determined after drying at 55°C for 96 h.

The intestine (with contents) was removed from the posterior end of the stomach to the beginning of the cloaca, the gonad from its point of attachment to the gonoduct, and the respiratory tree from its point of attachment to the cloaca. Intestinal and gonadal indices were calculated as the ratio of their wet mass to dry body wall mass (this permitted an examination of the intestinal contents and gonadal histology), whereas the respiratory tree index was calculated as its dry mass relative to the dry body wall mass. For each collection date, the various indices were determined for 15 males and 15 females, ranging from 25 to 34 g in dry body wall mass (equivalent to 5.7–6.1 cm in the distance from the mouth to the anus).

In order to compare them with the evolution of the gonadal indices, seasonal changes in the diameter of the gonadal tubules were quantified as follows: The gonads were spread out in a shallow container, and the tubule diameter was measured at random points with a binocular scope (12×). Fifteen measurements for each of 15 males and 15 females were made for each sampling date.

Gametogenesis

Gonads from preserved individuals were removed and transferred to Bouin's fixative for four weeks and then processed according to standard embedding technique (Junqueira *et al.*, 1986). To determine the variation due to differences in tubule size, separate examinations were made of small (<1.9 mm) and large tubules (>1.9 mm). To prevent the loss of tubule contents during embedding, the tubule sections were cut well beyond the segment selected for sectioning. For each individual, six 5 μm-microtome sections were cut from both the small and large tubules. These sections were first placed on gelatin coated slides (the gelatin was heated to 42°C) and then transferred

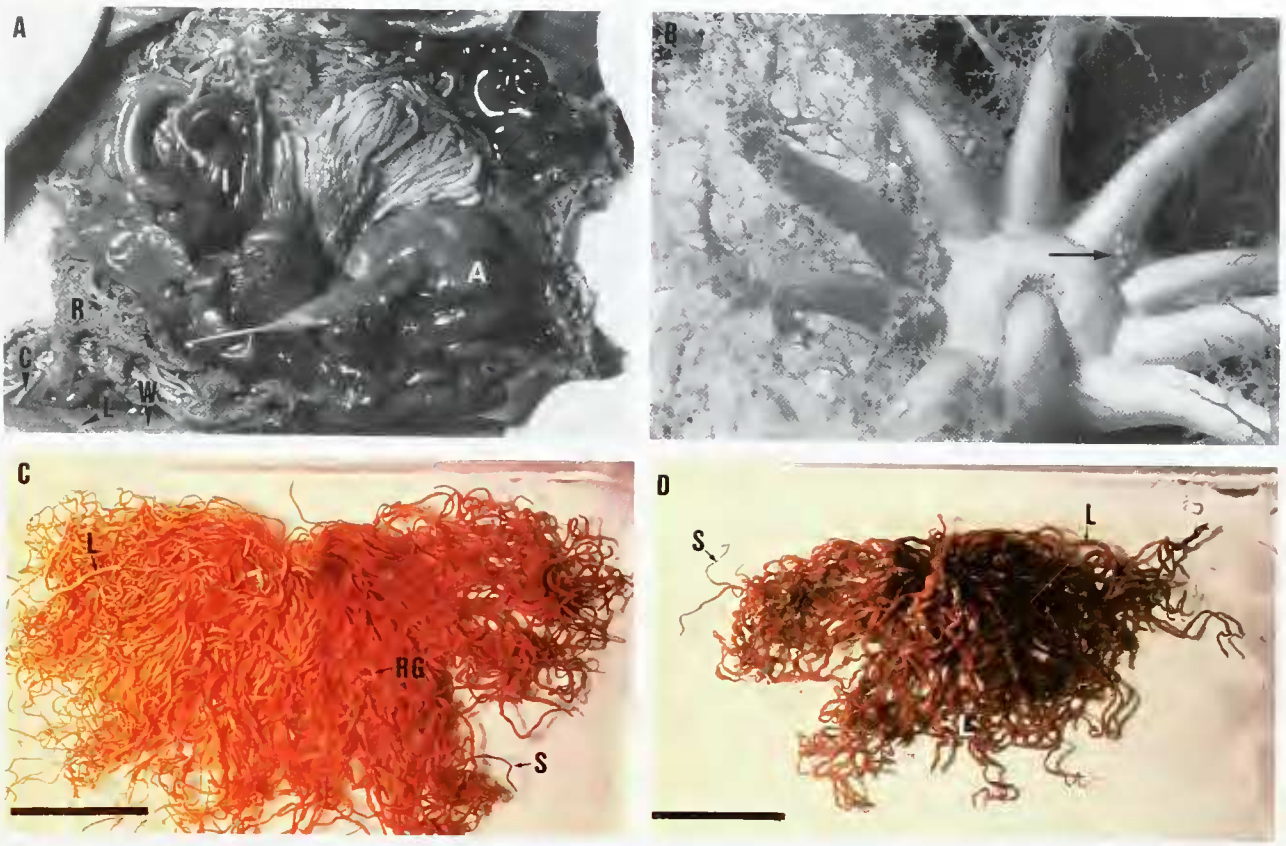


Figure 1. *Psolus fabricii.* (A) Dissected male showing the respiratory tree (R), intestine (I), body wall (W), aquapharyngeal bulb (A), cloacal muscles (C), longitudinal muscles (L), and testis (G). (B) Photograph of the mouth region showing the feeding podia, one of which is in the mouth and the others extended, and papillae surrounding the gonopore (arrow). Photographs of a testis (C) and an ovary (D) just after spawning showing the large tubules (L), small tubules (S), and tubules with swellings containing residual gametes (RG). The horizontal bars in photographs C and D represent 50 mm.

to an oven at 37°C for 1 h. This technique usually prevented the breaking of the fragile tubules and the loss of gametes. The slides were stained with eosine and hematoxylin, as described by Galigher and Kozloff (1971), and good resolution of the various cell types was achieved. A second series of slides was stained with the periodic acid-Schiff (PAS) reaction (Humason, 1981) to identify polysaccharides (glycogen).

Gonadal development was classified into five stages (post-spawning, recovery, growth, advanced growth, and mature stage) that were adapted from the earlier studies of holothurians (Tanaka, 1958; Costelloe, 1985; Cameron and Fankboner, 1986). For each male, we made 15 random measurements of the thickness of the gonadal tubule wall from slides of both small and large tubules. Only intact areas were used in this assay. For each female and for both small and large tubules, we determined the diameters of 200 relatively unbroken oocytes that showed a well-centered germinal vesicle.

Size of the gonad at sexual maturity

A sample of 132 individuals was collected on 4 May 1988. For each of these individuals, we measured the gonadal index and made histological sections to determine whether mature gametes were present. We also determined the total number of tubules in each individual, as well as the length of 15 randomly selected tubules, and the length of the intestine.

Environmental factors

Continuous temperature measurements at the study site at Anse à Robitaille were made during most of our study using a Peabody Ryan thermograph placed at 10 m in depth. Data on day length and minimum daily sunshine were obtained from the weather station at the Québec Airport (Environment Canada, Atmospheric Environment Service). Data on freshwater run-off were provided by Environment Canada (Climatologic Services) by

using the combined discharges from the Montmorency, Bastiscan, Saint-Anne and Chaudière rivers.

Phytoplankton cells abundance control the spawning of a number of marine invertebrates in the Estuary, but regular phytoplankton measurements could not be made during our study. As an indirect signal of the spring phytoplankton bloom, we determined, in 1989, the time of spawning in the green sea urchin *Strongylocentrotus droebachiensis*. This species spawns when the adults detect the rapid growth of phytoplankton during the spring bloom (Himmelman, 1981; Starr, 1990; Starr *et al.*, 1990, 1992). Thus, from March to August, when spawning was anticipated, gonadal indices (percentage gonadal mass) were determined for 15 adult urchins (4.0–6.5 cm in diameter) of both sexes at each sampling date.

We used two approaches to examine seasonal changes in the intestinal contents of *Psolus fabricii*. First, for each date, the contents of the first centimeter of the intestine of each of the 30 individuals were suspended in 5 ml of 10% formalin, and the various types of undecomposed organisms present in a 1 ml subsample were identified and counted with a hemacytometer. Large cells were examined under white illumination, and the presence of small phytoplankton cells was determined by the fluorometric method of Yentsch and Menzel (1963). Second, the contents of the following 30 g portion of the digestive tract was emptied into a Petrie dish, examined with a binocular scope, and the proportion of living phytoplankton cells (green in color) to non living materials (decomposed cells and inorganic materials) was estimated. The phytoplankton cells in the first centimeter of the intestine seemed virtually undigested (green in color and intact).

Buoyancy of oocytes

Forty oocytes were collected from five mature females (measuring 25–34 g in dry body wall mass) collected on 14 July 1990. The oocytes were placed in natural seawater to provoke breakdown of the germinal vesicle.

The oocytes were then placed in a 500 ml graduated cylinder (5 cm in diameter) at 7–8°C and the rate of upward vertical movement was recorded (Fig. 8). This movement was taken as a measure of buoyancy.

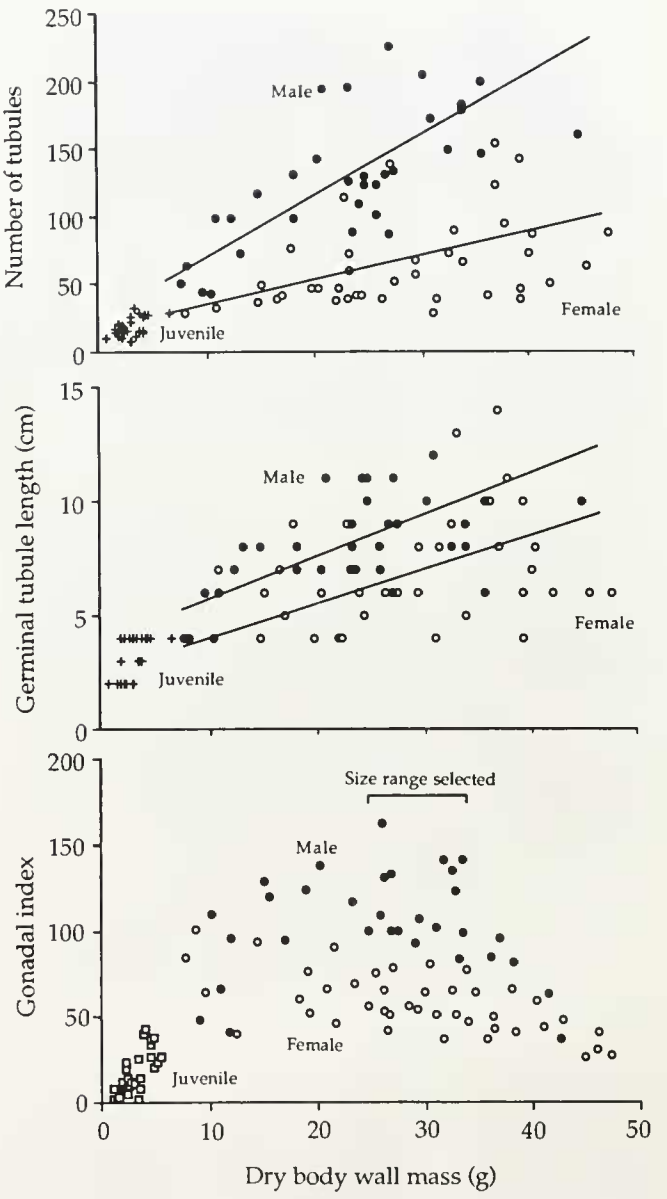


Figure 2. *Psolus fabricii*. The relation of the number of germinal tubules (A) and of germinal tubules length (B) to dry body wall mass for juvenile and adult males and females in May 1988 (n = 132). (C) The relation of the gonadal index to dry body wall mass (n = 132) in May 1988. The bracket indicates the size range used for gonadal index determinations.

Results

Gonadal morphology and size at sexual maturity

The gonads of *Psolus fabricii* consist of a large number of rarely branched germinal tubules distributed throughout the perivisceral cavity (Fig. 1C, D). The tubules join at a single gonoduct which exits through a gonopore located between the feeding podia (Fig. 1B). The number of tubules is greater for males than for females (Fig. 2A, Z test, $P < 0.01$, Tessier's slope analysis, Tessier, 1948), whereas tubule length does not vary significantly between the sexes (Fig. 2B, Z test, $P > 0.05$). In some very large males and females, the terminal ends of some tubules were necrotic, and at times these ends were found floating free in the coelomic fluid.

The size of the gonad at sexual maturity was determined from the 132 individuals collected on 4 May 1988 (Fig. 2C). Gonadal tubules were present in every individual examined that had a dry body wall mass of at least 1.2 g

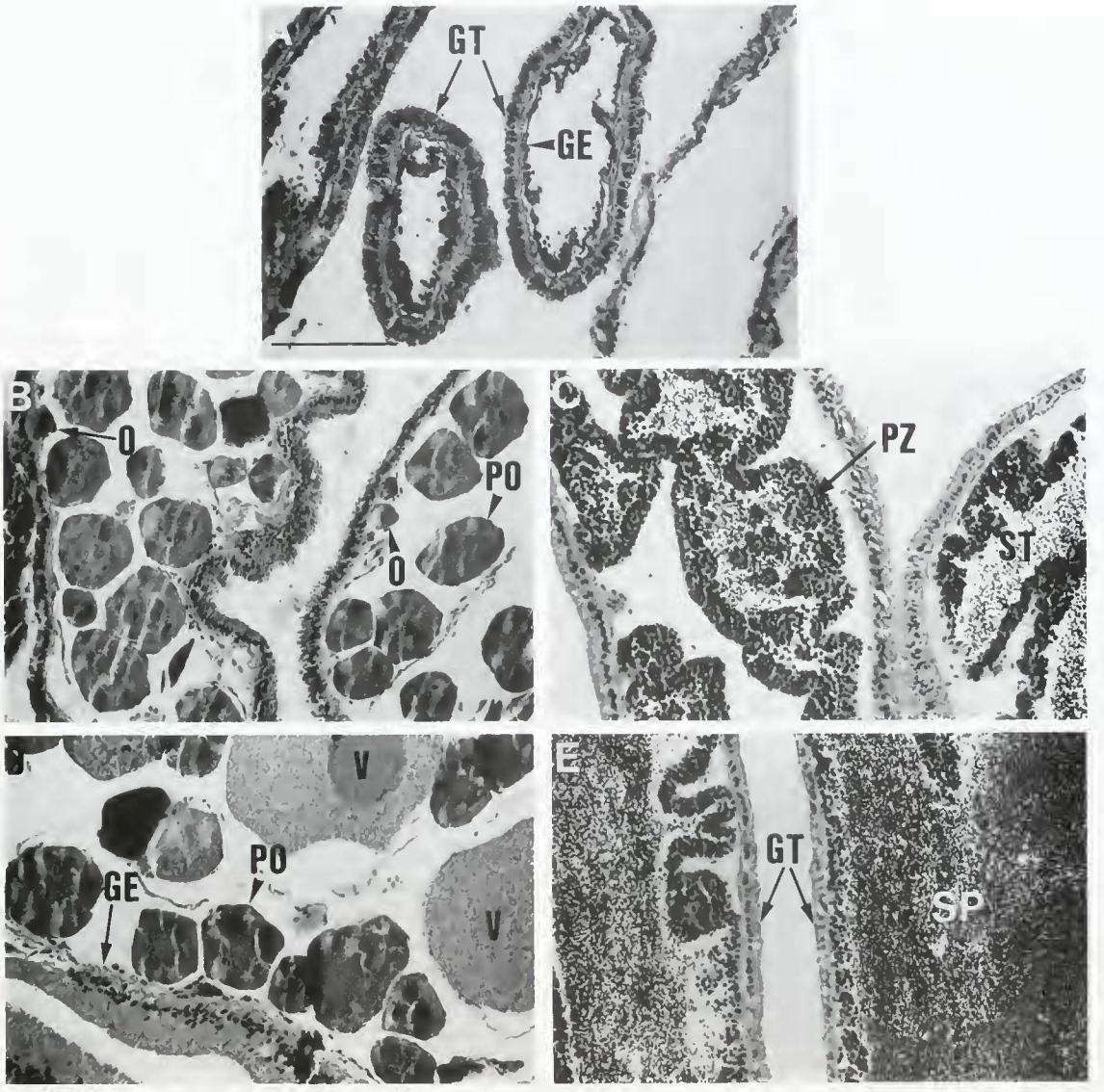


Figure 3. *Psolus fabricii* Light micrographs of sections of gonads of juveniles. (A) Immature gonad (from an individual weighing <5.5 g in dry body wall mass) showing the germinal epithelium (GE) and an absence of identifiable precursor cells for two germinal tubules (GT); (B) Immature female (between 5.6–10 g) with oogonia (O), primary oocytes (PR), but an absence of more advanced stages; (C) Immature male (between 5.6–10 g) showing the proliferation zone (PZ) and the lumen containing only a few spermatozoa (SP); (D) Young female (≈10 g) showing the germinal epithelium (GE), primary oocytes (PO) and a few vitellogenic oocytes (V); (E) Young male (≈10 g) showing two distinct germinal tubules (GT) and numerous spermatozoa (SP) in the lumen. The horizontal bar in photograph A represents 800 μm and applies to all of the photographs.

(equivalent to ≈0.7 cm in distance mouth-anus). The relative size of the gonads increased sharply as the dry body wall mass rose from 3 to 10 g (Fig. 2C). The size of testis and ovary overlapped greatly up to a body wall mass of ≈15 g, but the testis was generally larger than the ovary for larger individuals (Kruskal-Wallis, analysis of variance, $P < 0.01$, followed by a non-parametric multiple-range test, $P < 0.05$; Sokal and Rolph, 1981). The variation in gonadal size was relatively small between 20 and 34 g (5.5 to 6.1 cm); beyond that range the relative gonadal size dropped.

Histological preparations showed that only undifferentiated precursor cells are present along the germinal epithelium of individuals weighing <5.5 g (≈3.2 cm) (Fig. 3A). The sex of larger individuals could be identified by the presence of oogonia and young oocytes in females, and spermatogonic stages in males (Fig. 3B, C). Beginning at 6.5–7.9 g (4.0–4.6 cm), the sexes were readily recognized

from gonadal smears (Fig. 2C), although histological examination showed that only individuals weighing >10 g (≈4.8 cm) contained mature gametes with the same morphology and reaction to PAS and hematoxylin and eosine as for very large individuals (Fig. 3D, E). Immature gonads were cream in color, whereas the mature testis was pink, and the mature ovary reddish brown (individuals > 10 g). Thus, *Psolus fabricii* starts to produce mature gametes at 10 g, but the gonads only attain a plateau in size at 15–20 g (Fig. 2C). Individuals weighing 25 to 34 g showed no variation due to body size and were used in following the gonadal index cycle.

No significant departure from a sex ratio of 1:1 was observed in any of the samples, and the ratio for all of the samples together was 595 males to 607 females (df = 14, $\chi^2 = 1.44$, $P > 0.05$). No external differences were observed between the sexes, and hermaphrodites were not encountered.

Seasonal changes in body component indices

Throughout the study, the mean gonadal index of males was more than twice that of females (Kruskal-Wallis, $P < 0.01$), and on no date did the maximum value for any given female attain the minimum observed for the males (Fig. 4). Nevertheless, the two sexes showed parallel seasonal cycles in gonadal size. The index for males and females dropped significantly between 14 May and 12 June 1988 (Kruskal-Wallis, $P < 0.01$), suggesting the release of gametes. The gonads showed no further significant change in size until the end of the following winter (in March 1989 for males and April 1989 for females), when a significant growth was evident (Kruskall-Wallis, $P < 0.01$). Both testicular and ovarian indices attained a peak in mid July 1989 and then dropped abruptly by 5 August 1989, suggesting a second spawning.

The diameter of the germinal tubules showed a similar pattern, although the annual cycle was more pronounced (Fig. 4). In both years, the mean diameter attained a maximum just before spawning (higher in 1989 than in 1988) and dropped precipitously during spawning (Kruskal-Wallis, $P < 0.01$). The decrease was by ≈24% in 1988 compared with ≈80% in 1989. The diameter of any given tubule was relatively uniform, except after spawning when some tubules had swollen sections containing unspawned gametes. The mean diameter of male tubules was consistently larger than that of females during May through August (Kruskal-Wallis, $P < 0.01$), but not during autumn and winter (Kruskal-Wallis, $P > 0.05$). In spite of the distinct seasonal pattern in mean tubule size, extremes in tubule size were always evident, and every gonad contained tubules ranging from small to large.

The 1989 spawning was also observed directly by persons diving in our study site on 22 July (Normand Piché and Andréa Cantin, pers. comm.). Numerous females were seen releasing eggs. Spawning was probably widespread and massive in the lower St. Lawrence Estuary because on 25 and 26 July 1989 other divers observed an abundance of *Psolus fabricii* oocytes and embryos throughout the first 3 m of the water column over a long section (≥5 km) of the southern side of the Estuary near Rimouski (Lucie Bossé, Institut Maurice-Lamontagne, pers. comm.).

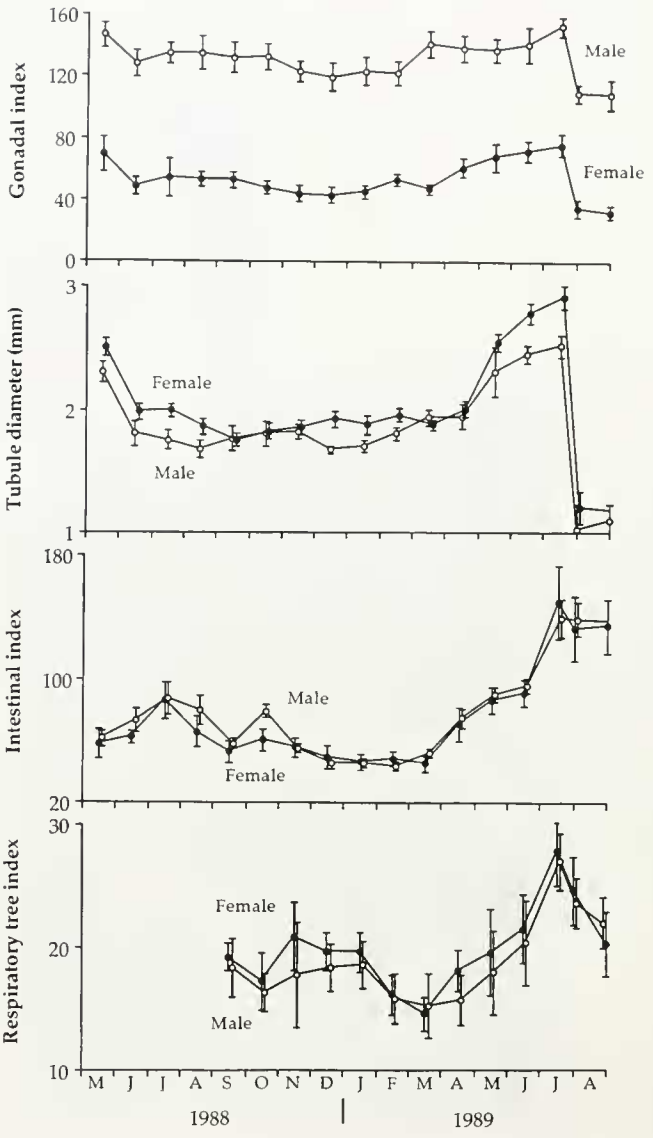


Figure 4. *Psolus fabricii.* Seasonal changes in the mean gonadal, intestinal and respiratory tree indices and in the diameter of the germinal tubules for males and females from May 1988 to August 1989. Vertical lines indicate the 95% confidence intervals.

Variations in the intestinal index were largely attributable to changes in the intestinal contents: the mass of the wall of the first centimeter of the intestine varied by <2% throughout the study (and no significant seasonal

changes were detected, Kruskal-Wallis, $P > 0.05$), whereas the mass of its contents varied by $\approx 37\%$ (Kruskal-Wallis, $P < 0.01$). The intestinal index showed intermediate values during the summer of 1988, a minimum between December 1988 to March 1989, and then a sharp increase to a maximum in July 1989. The maximum attained in 1989 was much greater than in 1988 (Fig. 4). The respiratory tree decreased during the winter of 1988–89, started to growth in April 1989, and reached a peak in mid July 1989 (Fig. 4).

Female reproductive cycle

Oogenesis. The development of gametes in *Psolus fabricii* was transversal, starting at the surface of the germinal epithelium and progressing towards the lumen of the tubule. In addition, it proceeded in a relatively uniform fashion along all surfaces of any tubule. Along the surface of the germinal epithelium, oogonia occurred in groups at numerous points, whereas primary oocytes (<100 μm) were dispersed. The small oocytes (<250 μm) were surrounded by follicular cells which persisted until spawning. The germinal vesicle is central and also persisted until spawning. The small oocytes have a PAS-negative basophilic cytoplasm which becomes slightly PAS-positive upon attaining 300 μm (indicating the beginning of glycogen accumulation), and increasingly positive as vitellogenesis progressed. The morphology and histological staining indicated that the oocytes were mature at ≈ 800 μm, although they could attain up to 1400 μm in diameter. Nutritive phagocytes were associated with the tubules that had released gametes. For both males and females, the tubule wall became more and more PAS-positive after spawning until January-February, and then progressively PAS-negative until the following spawning period. The following five stages of oogenic development were used to quantify the seasonal oogenic changes (Fig. 5).

(1) Post-spawning (Fig. 5A). The gonadal tubule wall is thin and extremely convoluted. Although some residual or unspawned oocytes, measuring 400–800 μm, remained in the tubules, the majority of oocytes measured <300 μm and are generally PAS-negative. Striking elongated empty areas are seen in the tubules, suggesting the passage of oocytes along the length of the tubule during spawning. Nutritive phagocytes begin to appear and are always inside of the follicular cells that surround the residual oocytes. The follicular cells around the residual oocytes were degenerated.

(2) Recovery (Fig. 5B, C). The gonadal tubule wall is very thick. The germinal epithelium is convoluted, and beds of small oocytes (<200 μm in diameter) are present along the epithelium. Nutritive phagocytes are closely associated with nearly all of the residual oocytes and the follicular cells are poorly defined.

(3) Growth (Fig. 5D). The thickness of the tubule wall reaches its maximum. Along the surface of the germinal epithelium, many small oocytes (<200 μm, PAS-negative) and some previtellogenic oocytes (300–600 μm, PAS-positive) are present and nutritive phagocytes are virtually absent.

(4) Advanced growth (Fig. 5E). The tubule wall is thinner, and the diameter of the tubules is increased. In the lumen of the tubules, well-defined follicular cells are associated with large PAS-positive previtellogenic (400–600 μm) and vitellogenic (>600 μm, PAS-positive) oocytes. The vitellogenic oocytes are reddish orange. Numerous small oocytes (<400 μm) are present along the germinal epithelium.

(5) Mature (Fig. 5F). The tubules are highly dilated, their walls thin and not convoluted, and they are almost completely filled with mature oocytes (>800 μm). Each oocyte contains one to four nucleoli and a well-defined germinal vesicle, which occupies 30–50% of the surface of the oocyte in the histological preparations. Immature oocytes are virtually absent.

Seasonal changes in the oogenesis. Advanced oogenic stages (advanced growth and mature stages) predominate in the large tubules, and earlier stages (post-spawning, recovery and growth stages) in the small tubules (Fig. 6). Nevertheless, both categories of tubules showed a seasonal pattern that is correlated with the gonadal index cycle. In the large tubules, the post-spawning and recovery stages are almost always absent, and the major evidence of the June 1988 and July 1989 spawnings was the decrease in the mature stage. In contrast, the mature stage in the small tubules is rare before spawning and absent in other periods, and the major evidence of spawning was a sharp increase in the post-spawning stage (from 0 to 80%). These observations suggest that the release of mature oocytes during spawning transforms large fecund tubules into small tubules in post-spawning condition. This change coincides with a sharp drop in the mean diameter of tubules (Fig. 4). Following spawning there is a period of inactivity until mid January; then oocyte development resumes. In small tubules, a progressive increase in the frequency of the growth stage occurred between January and July 1989 coincident with a decrease, first in the post-spawning stage, and then in the recovery stage. Meanwhile, the large tubules show an increase in the advanced growth and mature stages (Fig. 6).

Size of oocytes. The seasonal pattern in the size structure of oocytes varies markedly between large and small tubules (Fig. 7). In large tubules, a striking change in the oocyte population occurred during the 1988 spawning. Prior to spawning, most oocytes measured >800 μm, whereas after spawning in June 1988, 500–700 μm oocytes predominated. Following this, the oocyte population in the large tubules was stable. Then in January 1989, renewed oocyte

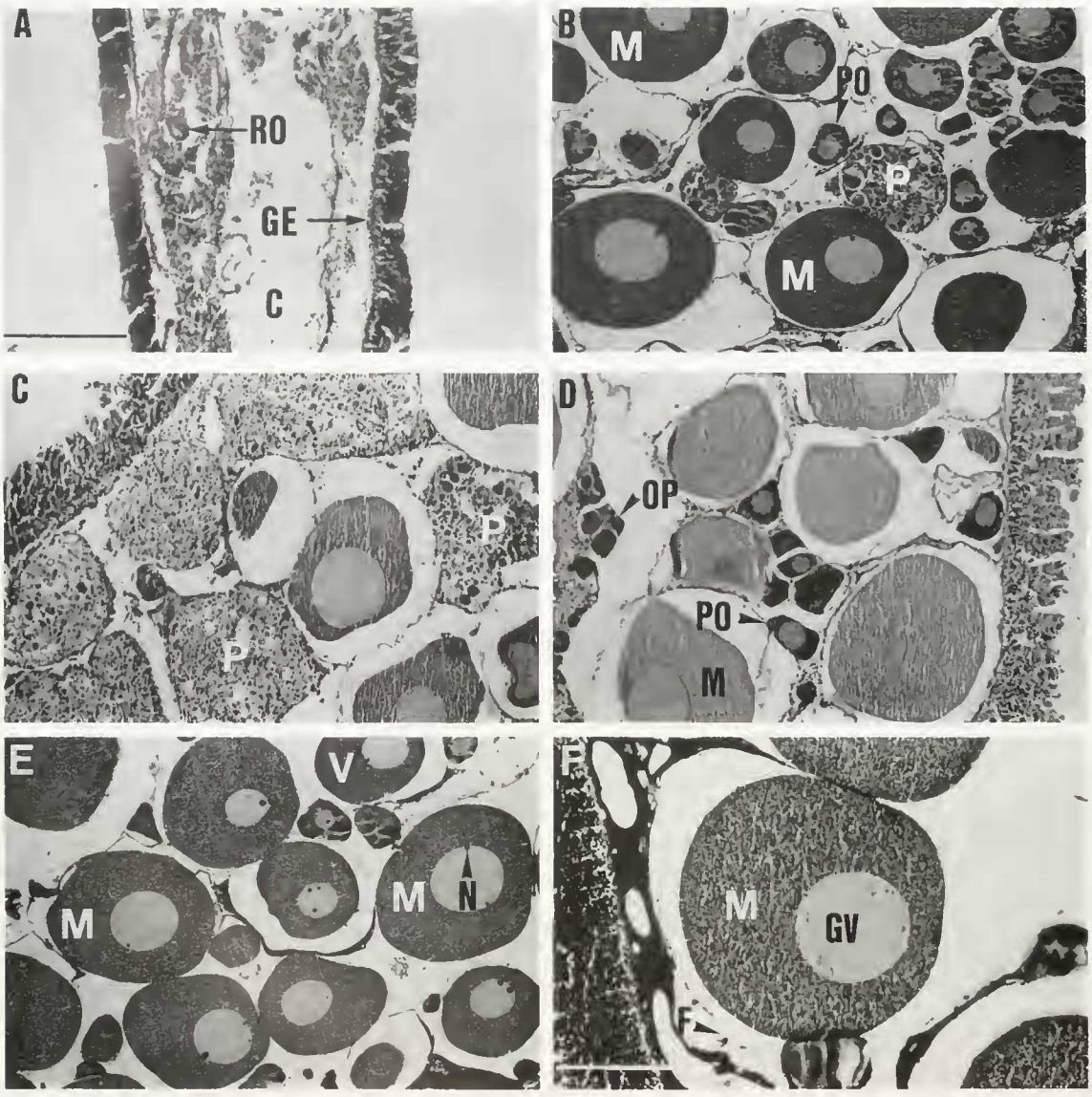


Figure 5. *Psolus fabricii.* Light micrographs of ovarian sections illustrating the oogenic cycle. (A) Portion of a post-spawning ovary showing the germinal epithelium (GE), residual oocytes (RO), and a channel created by the expulsion of eggs during spawning (C); (B) Early recovery stage showing primary oocytes (PO), mature oocytes (M), and nutritive phagocytes (P) surrounded by follicular cells. (This section was across a swelling containing residual gametes in a spent tubule); (C) Late recovery stage showing an abundance of nutritive phagocytes (P); (D) Growth stage showing sites of oogonial proliferation (OP), primary oocytes (PO), and mature oocytes (M); (E) Advanced-growth stage showing an abundance of both vitellogenic oocytes (V) and mature oocytes (M) with nucleoli (N); (F) Mature stage showing large mature oocytes (M) containing the germinal vesicle (GV) and surrounded by follicular cells (F). The bar in photograph A represents 800 μm and applies to photographs B, C, D and E, whereas the bar in photograph F represents 400 μm.

growth was evident, and the predominant mode of oocytes attained a peak of 1100 to 1300 μm in mid July 1989. These mature oocytes largely disappeared during the July 1989 spawning, and on 5 August the modal oocyte class was again 500–700 μm. Although the loss of large oocytes (>800 μm) during spawning was expected, the presence of a strong cohort of intermediate oogenic stages (500–700 μm) in the large tubules after spawning seemed at first surprising. This was because spawning transformed the large tubules into small tubules. Thus, the small tubules in June 1988, which were characterized by a strong mode of oocytes measuring <300 μm, were probably those which had just spawned. They contained residual oocytes that were being attacked by nutritive phagocytes, whereas

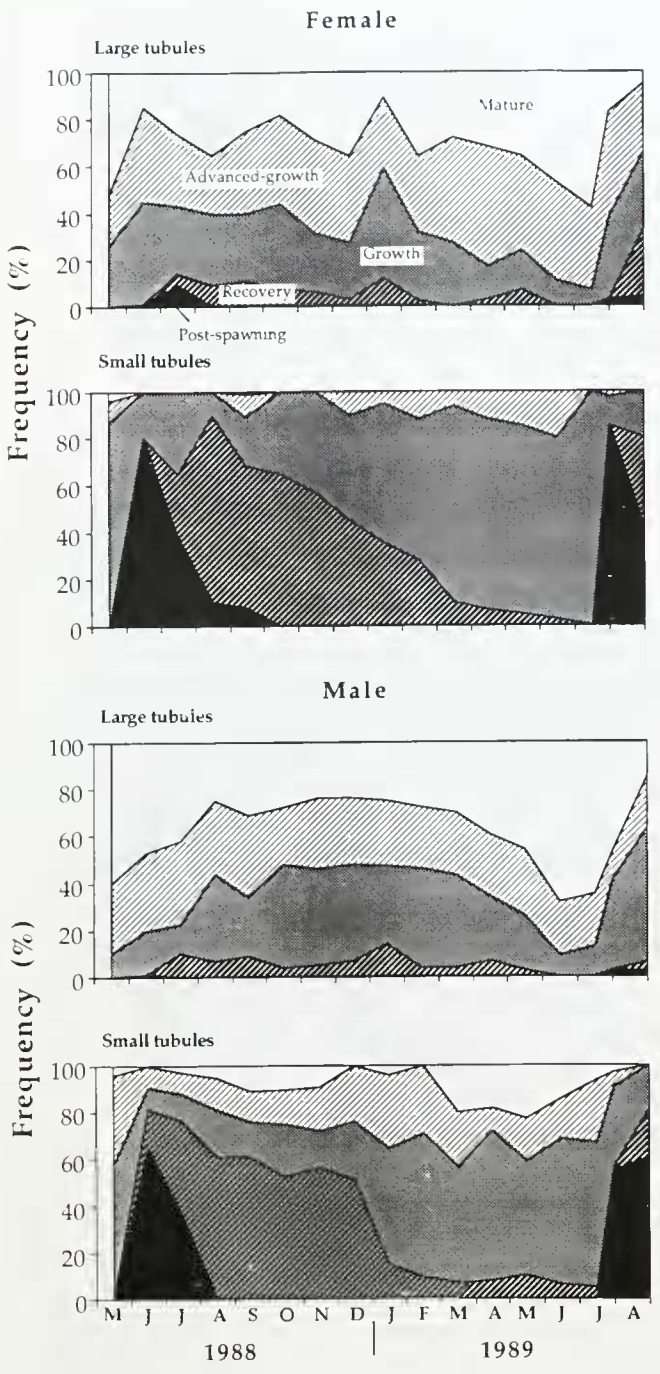


Figure 6. *Psolus fabricii.* Relative frequency of different gametogenic stages (as defined in the Materials and Methods section) in small and large, female and male, tubules for the period from May 1988 to August 1989.

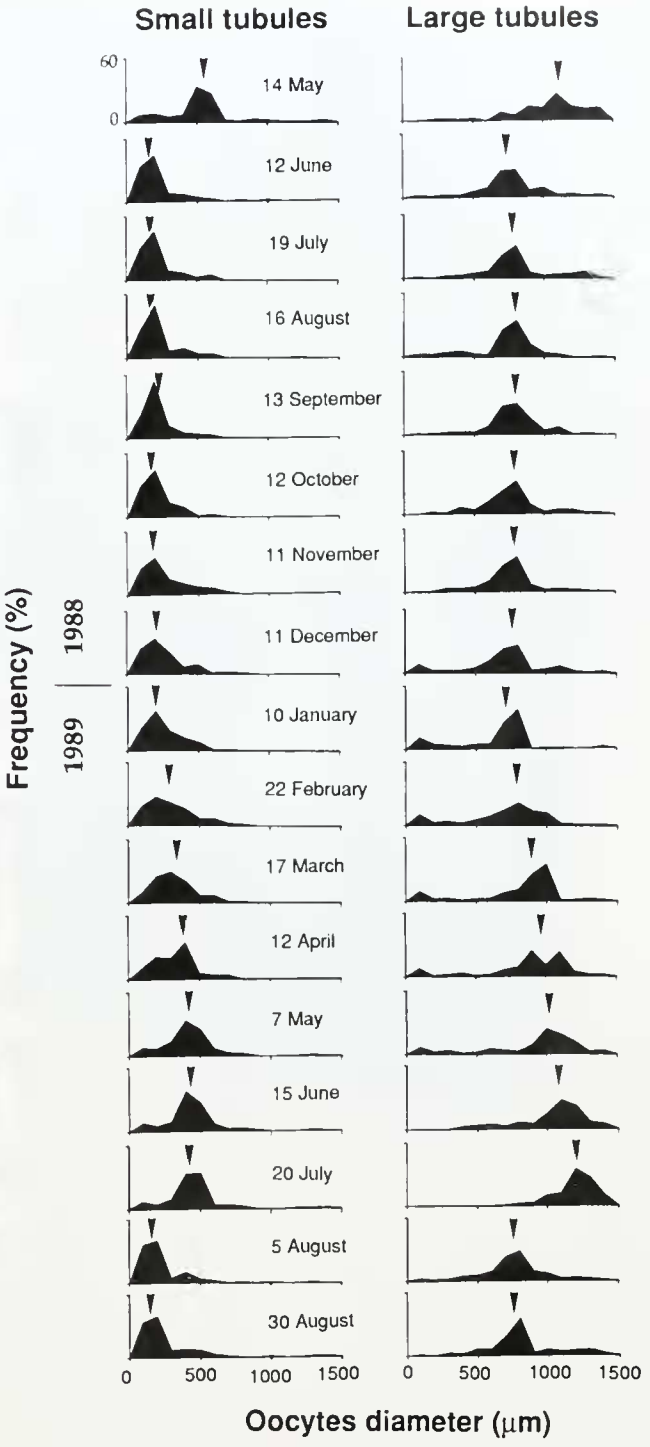


Figure 7. *Psolus fabricii.* Oocyte diameter distributions for small and large tubules for the period from April 1988 to August 1989. For each distribution the vertical axis is from 0 to 60% and the mean oocyte diameter is indicated by an arrow.

at the same time, the large tubules contained an abundance of intermediate stages, and nutritive phagocytes were rare. Most oocytes in the small tubules in mid May 1988 measured 400–600 μm, whereas those in the large tubules in June 1988 measured 600–800 μm. The similarity in size distributions for these two samples suggested the transition from small to large tubules during spawning. Following spawning, until mid December 1988, the oocyte

distributions for the small tubules showed little change. However, a marked increase in the total number of cells per surface of germinal tubule was noted in January 1989. Thereafter, oocyte size and number progressively increased in the small tubules until 20 July 1989 when the size structure was virtually identical to that of the small tubules prior to spawning in 1988. The sharp reduction in >800 μm oocytes in the large tubules during the 1989 spawning closely followed the changes in the large tubules during the 1988 spawning.

Buoyancy of oocytes. Thirty three of the oocytes showed a positive floatability which clearly increased with diameter (Spearman rank correlation coefficient, r = 0.67, df: 32, $P < 0.01$). This indicated that 1.2 mm oocytes would move upward at a rate of 20–30 mm · min $^{-1}$. The other four oocytes showed a slightly negative floatability, and we suspect that they were damaged (possibly the egg membrane was not intact) (Fig. 8). These observations indicate that spawned eggs will move to the surface of the water column. This agrees with the abundance of developing *Psolus fabricii* embryos near the surface, as observed by divers during the 1989 spawning.

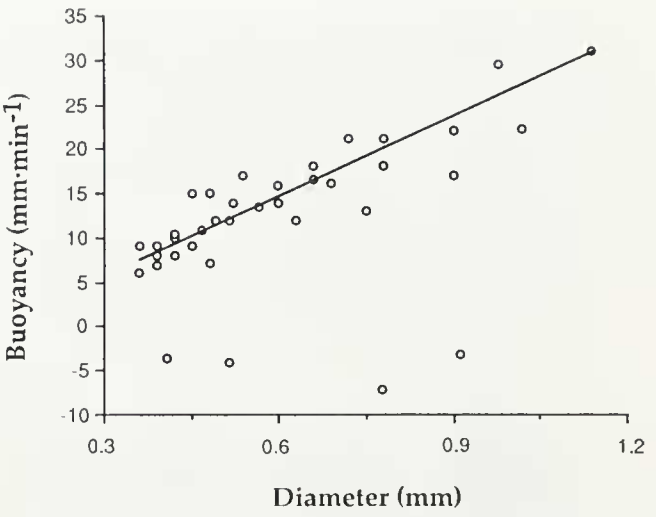


Figure 8. *Psolus fabricii* Relation of buoyancy to diameter for oocytes dissected for mature females and activated by being placed in seawater. The regression line is based only on oocytes with positive buoyancy.

Male reproductive cycle

Spermiogenesis. The following five stages of spermiogenesis are used to quantify the seasonal changes in the small and large tubules (Fig. 9).

(1) Post-spawning (Fig. 9A). The thickness of the tubule wall is at its minimum. In the sections, we observed elongated empty areas along the length of the tubules, suggesting the passage of gametes during spawning. A few residual spermatozoa are present, and no proliferating zone (containing spermatogonia, spermatocytes and spermatids) was present.

(2) Recovery. The tubule wall is extremely thick and highly convoluted. The tubules contain small quantities of spermatozoa and scattered nutritive phagocytes.

(3) Growth (Fig. 9B, C). The gonadal tubule wall is beginning to decrease in thickness but is still convoluted. Spermatogonia are abundant along the surface of the germinal epithelium. Progressing towards the lumen, there is a layer of spermatocytes, one of spermatids, and finally a small number of spermatozoa in the lumen.

(4) Advanced growth (Fig. 9D, E). The tubule wall is thinner and slightly convoluted, and the lumen is filled with spermatozoa.

(5) Mature (Fig. 9F). The tubules are stretched to their maximum diameter and completely filled with spermatozoa. The tubule wall is nearly smooth, and earlier spermatogenetic stages are absent.

Psolus fabricii spermatozoa are flagellated with a round head measuring 5–6 μm. Microscopic observation of a sperm suspension in seawater, just prior to spawning, revealed a low motility of the spermatozoa.

Seasonal changes in spermatogenesis. In May and June 1988 and again in July and August 1989, advanced stages (advanced growth and mature stages) were found in >85% of the large tubules, whereas earlier stages (post-spawning, recovery and growth stages) predominated in the small tubules (Fig. 6). The most striking evidence of the spawnings in June 1988 and August 1989 was the abrupt appearance of post-spawning stages in the small tubules. The tubules classified as large after spawning were characterized by an abundance of early spermatogenetic stages and few spermatozoa. These observations suggested, first, that the release of spermatozoa from the large tubules during spawning diminish their size, so that after spawning they were considered as small tubules. Moreover, the tubules that were selected as large were those that had recently attained a diameter of 1.9 mm (the lower limit for large tubules). Thus a pattern parallel to that observed for the females was found. The post-spawning stage, found only in the small tubules, disappeared by late summer and was replaced largely by the recovery and growth stages. Subsequently, the advanced growth and mature stages became more common and attained a peak a few month prior to spawning.

Thickness of the gonadal tubule wall. In males, the distributions for gonadal tubule wall thickness were virtually always skewed rather than being symmetrical (Fig. 10). Further, all size classes up to 140–160 μm were present in both small and large tubules throughout the study, except for four dates near the time of spawning, when the largest classes were absent. In both the small and large tubules, changes in the distributions followed an annual pattern. Just before spawning in May 1988, the mean thickness was 20–40 μm; after spawning it decreased

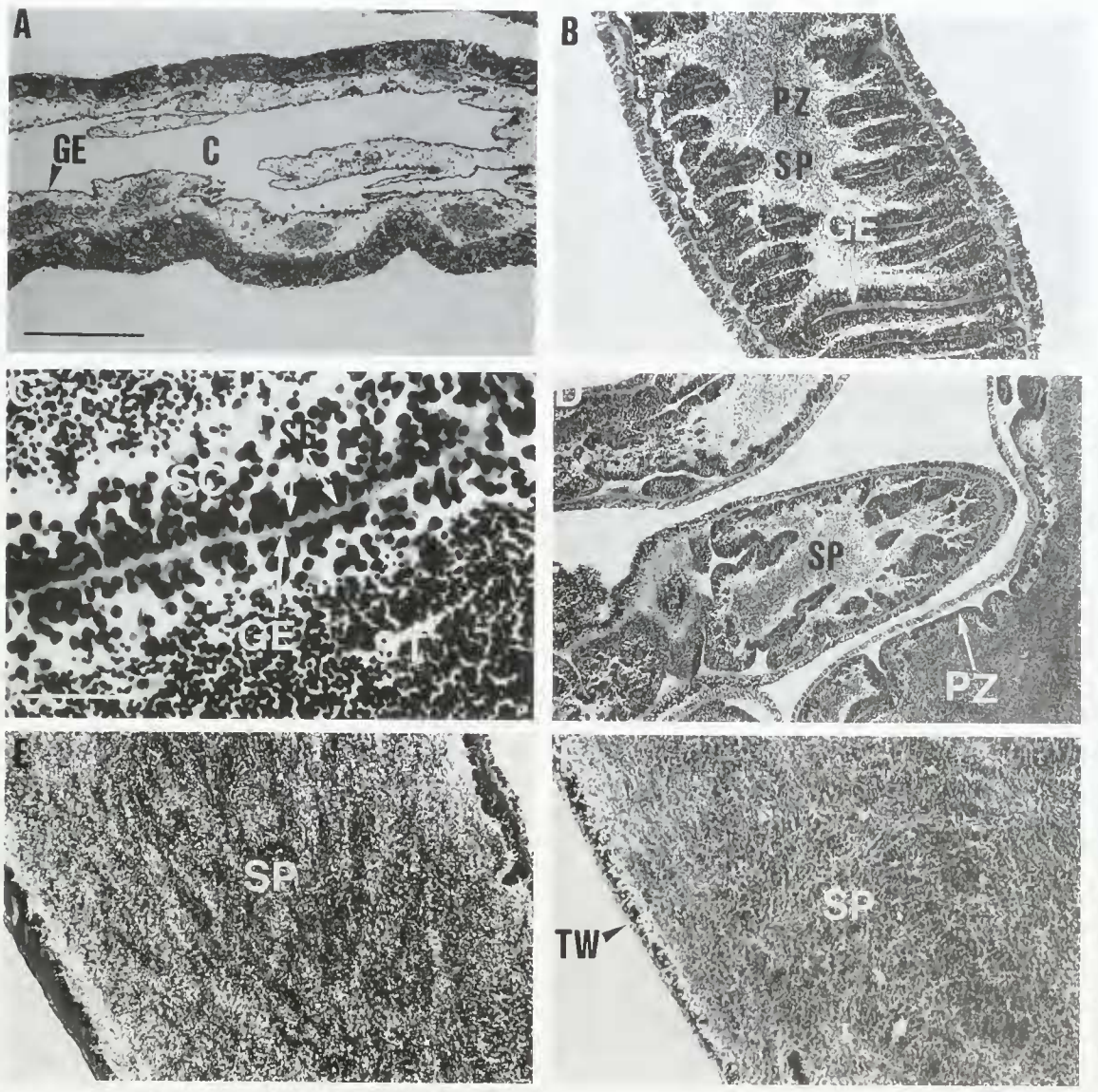


Figure 9. *Psolus fabricii.* Light micrographs of testicular sections illustrating the spermatogenic cycle. (A) Post-spawning testis showing the germinal epithelium (GE) and channels where sperm passed during spawning (C); (B) Growth stage showing the highly convoluted germinal epithelium (GE) and the proliferation zone (PZ); (C) Growth stage showing the germinal epithelium, spermatogonia (SG), spermatocytes (SC) spermatids (ST) and spermatozoa (SP) in successive layers progressing towards the lumen; (D) Early advanced-growth stage showing the proliferating zone (PZ) and spermatozoa (SP); (E) Late advanced-growth stage showing the thin gonadal tubule wall and an abundance of spermatozoa (SP); (F) Mature stage showing the thin tubule wall (TW), absence of the proliferation zone, and great numbers of spermatozoa (SP) in the lumen. The bar in photograph A represents 800 μm and applies to photographs B, D, E and F, whereas the bar in photograph C represents 300 μm.

slightly. Subsequently, the thickness of the tubule wall progressively increased, although the pattern varied depending on tubule size. Thus, the large tubules grew more rapidly and attained a peak ($\approx$120 μm) in November 1988, whereas the small tubules did not attain a peak ($\approx$140 μm) until February 1989. Subsequently, the size of the modal size class again decreased to 20–40 μm following the 1989 spawning (Fig. 10).

Environmental factors

Temperature. The mid May to mid June spawning, in 1988, coincided with the spring warming period, and temperatures attained about 5°C at the time of spawning (Fig. 11). However, temperatures fluctuated markedly during this period. An increase from 4 to 6°C was observed between 20 and 23 May 1988, and a drop of 6.7

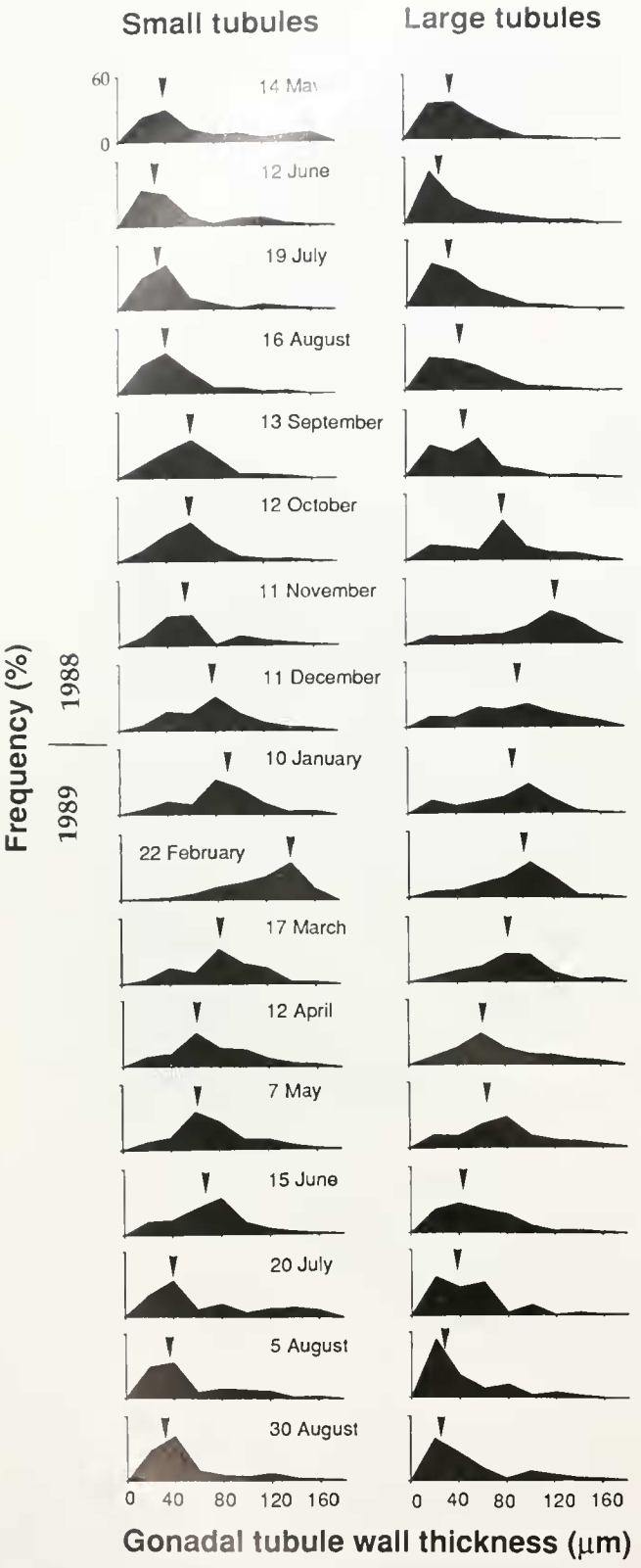


Figure 10. *Psolus fabricii.* Frequency distributions (10 μm size classes) of the thickness of the gonadal tubule wall for small and large tubules for males collected from May 1988 to August 1989. The arrows indicate the mean thickness for each sampling date.

to 4.4°C between 5 and 6 June. These variations were due to the semidurnal tides in the Estuary (Demers *et al.*, 1986). During 1988, the maximum temperature was reached in mid July, and the autumnal decrease began in late August.

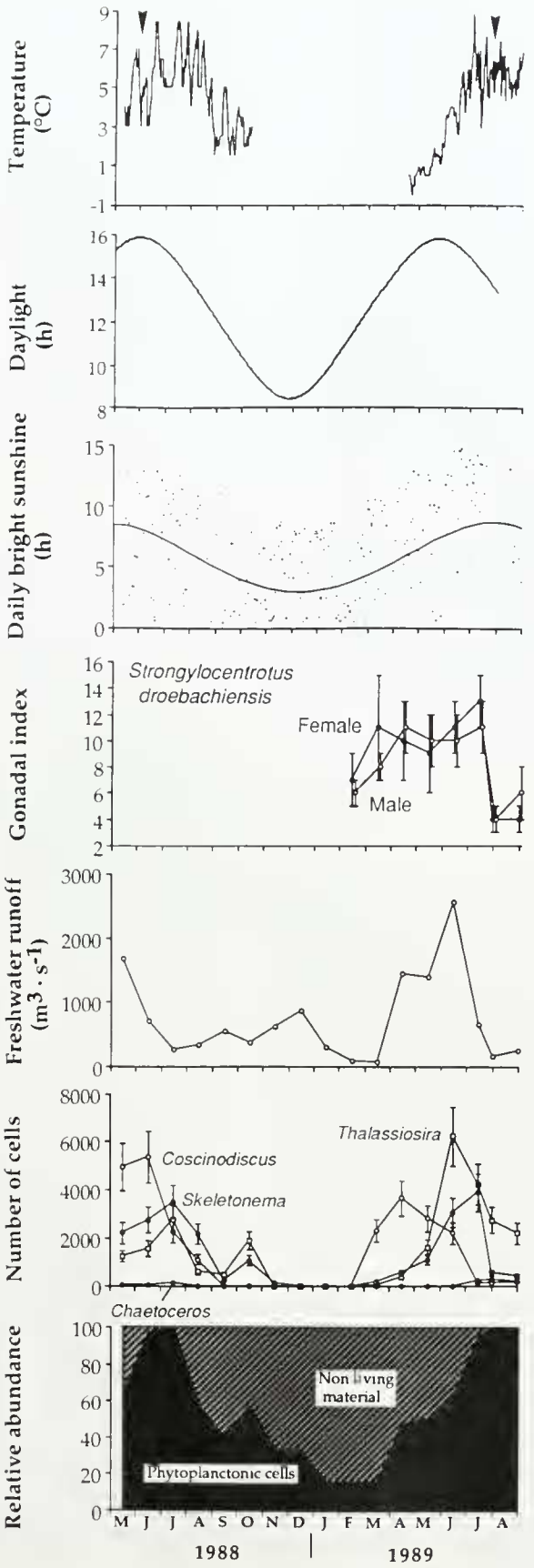
In 1989, warming began in March, attained a peak in late June, and then varied around 6°C until the end of the study (2–4°C daily fluctuations were frequent). The accelerated gonadal growth, which began in March or April, occurred at about the time that the vernal warming began. There was no spawning, either during the warming phase or during marked temperature variations in May and June; rather, spawning occurred in late July when temperatures were more stable. Thus, although spawning occurred at about the same temperature in the two years (5–6°C), the point in the temperature cycle was quite different (Fig. 11).

Photoperiod. In our study, the renewal of gametogenesis in January coincided with the period when day length and daily bright sunshine were beginning to increase, and the gonadal peak was attained at the photoperiod maximum (Fig. 11). Although *Psolus fabricii* spawned near the photoperiod maximum in both years, the 1988 spawning occurred at the beginning of this maximum and the 1989 spawning one month later, when photoperiod was just beginning to decline.

Freshwater run-off and the predicted timing of the phytoplankton bloom. The period during which freshwater run-off decreased in the Estuary was much later in 1989 (July) than in 1988 (late May) (Fig. 11). Nevertheless, spawning in both years coincided with this event, suggesting a relationship between the two. 1989 was an exceptional year in that the run-off was markedly greater and more delayed than in the five previous years. It was also unusual in that spawning of the green sea urchin *Strongylocentrotus droebachiensis*, a signal of the phytoplankton increase, was much later than in the previous years. The urchin spawned abruptly between 14 July and 5 August, exactly the same period during which *Psolus fabricii* spawned (Fig. 11). In contrast, when urchins were studied in the Estuary in the previous years, spawning occurred prior to mid June (Starr, 1990).

Intestinal contents. The intestine of adult *Psolus fabricii* contains two types of materials: (1) non living particles and (2) phytoplanktonic cells; the major species of plankton are the diatoms *Thalassiosira* sp., *Coscinodiscus* sp., *Chaetoceros* sp., and *Skeletonema* sp. (Fig. 11). During the autumn and winter, only 20–50% of the contents were phytoplanktonic cells, and this increased during the spring, attaining virtually 100% in the summer (Fig. 11).

A marked seasonal pattern was evident for the diatoms present in the intestines. Most cells in the first samples in May and June 1988 were *Coscinodiscus* sp., *Thalassiosira* sp., and *Skeletonema* sp., suggesting a diatom bloom at



this time. Subsequently, there was a progressive decrease to the winter minimum. In 1989, *Coscinodiscus* sp. showed an increase during March and April, and *Thalassiosira* sp. and *Skeletonema* sp. an increase in June (Fig. 11). The latter two species increased further to the highest level for the study in late July. This suggested an intensive bloom at this time. Whereas the above large diatom species typically occur in chains in the water column, they were always present as individual cells in the intestines. No animal structures were observed in *Psolus fabricii* intestines.

Discussion

Morphological comparisons with other holothurians

Non-branched germinal tubules, such as are found in *Psolus fabricii*, also occur in *Cucumaria lubrica* and *Ypsilothuria talismani* (Atwood and Chia, 1974; Tyler and Gage, 1983), but branched tubules have been reported in other holothurians (Atwood, 1974; Smiley and Cloney, 1985; Cameron and Fankboner, 1986; Tyler and Billett, 1987). The increase in the number of gonadal tubules with size in *P. fabricii* (Fig. 2A) is in contrasts with *Aslia lefevrei*, where tubule number is highly variable and not related to size (Costelloe, 1985).

Throughout the year, the gonad of *Psolus fabricii* is larger in males than in females, and this is primarily due to the number of tubules in the testis rather than to tubule length or diameter (Fig. 2A, B). This, together with the greater drop in gonadal mass during spawning (Fig. 4), suggests that males have a greater reproductive output. A larger male gonad is not a general holothurian characteristic, since the inverse is the case for *Stichopus californicus* (Cameron and Fankboner, 1986), and equal-sized gonads are reported for *Cucumaria pseudocurata* (Rutherford, 1973). A sex ratio of 1:1 has been reported for several holothurians in addition to *P. fabricii* (Cameron and Fankboner, 1986; Jespersen and Lutzen, 1971; Conand, 1982; Engstrom, 1982; Mosher, 1982), but numerous others have a ratio favoring males (Lawrence, 1987).

Necrotic fragments of germinal tubules as found in *Psolus fabricii* have previously been described for *Sticho-*

Figure 11. Seasonal variations in temperature, daylight, daily bright sunshine, and freshwater run-off, as well as the relative proportion of living and non-living materials in a ≈30 g portion of the intestinal mass and the absolute abundance of the four major phytoplankton species, in the first centimeter of the intestine of *Psolus fabricii*, during the period from May 1988 to August 1989. The gonadal index cycle of the urchin *Strongylocentrotus droebachiensis* was quantified in 1989, and the drop in the index between 20 July and 5 August suggests that there was a phytoplankton bloom at this time. The vertical lines indicate the 95% confidence intervals. The arrows above the temperature cycle indicate when *P. fabricii* spawned.

pus californicus by Cameron and Fankboner (1986), but only for males. In both sexes of *P. fabricii,* necrotic tubules were most common in large individuals and thus may be related to the decrease in relative gonadal size in very large individuals. Aerobic metabolism furnishes most of the energy requirements of echinoderms (Ellington, 1982; Lawrence and Lane, 1982; Shick, 1983; Féral and Magniez, 1985), and Hopcroft *et al.* (1985) demonstrate that 75% of oxygen required by *P. fabricii* (individuals weighing 80 g in wet mass) is obtained through the respiratory tree. Thus, the increase in the size of the respiratory tree as the gonads grow may indicate its role in supplying oxygen for gametogenesis (Fig. 4).

The follicular cells associated with developing oocytes in echinoderms supply nutriments to the oocytes and also control the environment around them (Hirai and Kanatani, 1971). In *Psolus fabricii,* the follicular cells are closely associated with the oocytes as they migrate into the lumen during maturation, and they may surround the oocyte during spawning as reported for *Cucumaria elongata* (Chia and Buchanan, 1969). This contrasts with *Stichopus californicus* in which the follicular cells stay attached to the epithelium as the oocytes migrate into the lumen (Smiley and Cloney, 1985). The clearly stratified spermatogenesis of *P. fabricii* (Fig. 9) contrasts with that in *Leptosynapta clarki* and *C. lubrica,* in which spermatogonia and spermatids are erratically distributed throughout the lumen (Atwood, 1973, 1974).

Gametogenesis

In *Psolus fabricii,* oogenesis begins with the production of precursor cells in the small tubules in January. Progressively through the winter and spring these cells are transformed into oogonia and primary oocytes, and by mid summer 400–600 μm cells are most abundant (Fig. 7). These stages in small tubules do not contribute to spawning. After spawning, some small tubules attain >1.9 mm, a diameter sufficient to be classified as large tubules. During the autumn, the oocyte distributions in the tubules classified as large, remain virtually static, indicating a period of inactivity; then in January, oocytes growth and tubule enlargement resumes. This growth continues until the following summer when most oocytes measure >800 μm and the tubules attain their maximum diameter (Fig. 4). Finally, the release of these large oocytes during spawning results in a drop in the size of the tubules. From the time of spawning until the following January, nutritive phagocytes are active in destroying the residual oocytes (Fig. 5).

Our study also indicates a prolonged spermatogenesis. As with oogenesis, it begins with the production of precursor cells in the small tubules in mid winter, although a prior accumulation of reserves in these tubules is indicated by the thickening of the gonadal tubule wall during the previous autumn (Fig. 10). In late winter and spring, as the thickness of the germinal wall decreases, spermatogonia, spermatocytes, and spermatids progressively accumulate in the tubules (Figs. 9, 10). Since these tubules contain only small amounts of spermatozoa, they probably do not participate in spawning. During and after spawning they progressively attain the size of large tubules. The major change after spawning in what are now the large tubules is the thickening of the gonadal tubule wall and this peaks in February (Fig. 10). At about the same time, the production of spermatozoa increases, and this amplifies until a peak just prior to spawning (Fig. 6). Finally, with the release of sperm during spawning, these large tubules become small; after spawning nutritive phagocytes become abundant. This is the first report of the testicular cycle taking longer than a year in holothurians.

Although the examination of large and small tubules indicates that gametogenesis is prolonged, studies with radioactive markers are needed to determine its duration precisely. Our observations suggest that the production of the majority of gametes begins in winter and terminates 15–18 months later (two summers later). In some tubules, however, this process may be much longer or, at times, shorter.

That gametogenesis in *Psolus fabricii* generally takes more than a year was revealed from the separate histological studies of small and large tubules. Smiley and Cloney (1985) and Smiley (1988) examined the gonads of female *Stichopus californicus* collected in different seasons. Their observations of three size groups of tubules, with the most advanced stages of oogenesis only being present in the largest tubules, similarly led them to conclude that oogenesis was a long process. In contrast to *P. fabricii,* the various sized ovarian tubules of *S. californicus* are not intermixed. Rather they are arranged in order, the large fecund tubules being located posteriorly. Smiley and Cloney (1985) report that the large tubules are completely reabsorbed once the oocytes are released. Based on these observations, they propose that the tubules are produced at the anterior of the gonad and migrate posteriorly as they increase in size and state of development. Reabsorption does not occur in *P. fabricii,* since a new group of oocytes is evident in the fecund tubules at the time of spawning and persists in spent tubules during the autumn when residual gametes are being phagocytised. Thus, the pattern of tubule and gamete production in *S. californicus* contrasts markedly with that in *P. fabricii.* Resorption of tubules has also been noted in *Mesothuria intestinalis* (Théel, 1901) and *Ypsilothuria talismani* (Tyler and Gage, 1983), but probably does not occur in *S. japonicus* (Tanaka, 1958) and three species of sea cucumber examined by Conand (1981).

Gametogenesis in another holothurian, *Aslia lefrevrei*, in the same family as *Psolus fabricii* (Dendrochirotida), follows still another pattern. The tubules are of uniform width, and gametogenesis follows an annual pattern that is highly synchronized amongst the tubules (Costelloe, 1985). For example, during numerous periods in the year, all of the tubules are at the same stage of gametogenetic development. As in *P. fabricii*, the tubules are not reabsorbed after spawning, the growth continues for a subsequent year, oocytes (<200 μm) appearing prior to spawning. The above observations indicate that the pattern of gametogenesis varies markedly even within closely related holothurian species. Future studies should therefore consider the pattern of production of the tubules as well as the gametogenesis within the tubules.

Control of gametogenesis

The active phase of gametogenesis in *Psolus fabricii* begins in January and continues until spawning. In small tubules of both males and females, early gametogenetic stages proliferate. And in the large tubules, oocyte growth increases in females, and more advanced spermatogenic stages are produced in males. This renewed gametogenesis occurs when water temperatures are near freezing (≈ -1°C, Therriault, 1973; Ouellet-Larose, 1973), and an increase does not occur until several months later (March-April). Further, food conditions are minimal as evidenced by the near absence of phytoplankton cells in the intestines. The first increase in phytoplanktonic cells in the intestine is in mid March (Fig. 11). The only notable environmental change during the mid-winter renewal of gametogenesis is the return to increasing photoperiod. In fact, virtually the entire gametogenetic period coincides with the period of increasing day length and daily bright sunshine, and peak maturity is attained at the maximal photoperiod. These observations suggest that an increasing photoperiod controls gametogenesis. Photoperiod has been experimentally shown to control gametogenesis in numerous taxa including echinoderms (Pearse *et al.*, 1986; McClintock *et al.*, 1990). *P. fabricii* occurs where annual changes in temperature and food availability are pronounced, certainly more so than the habitats of other echinoderms that have been used to study the photoperiod control of gametogenesis. Nevertheless, the activation of gametogenesis well before the increase of temperature and food from their annual minima suggests the potential importance of photoperiod for *P. fabricii*.

The increased growth in tubule diameter and gonadal mass observed in March or April (Fig. 4) suggests a second point that may be controlled by environmental factors. At this time, photoperiod has been increasing for several months, and the most notable environmental change is the first increase of phytoplanktonic cells in the intestines (Fig. 11). Phytoplankton are probably not abundant at this time, but abundant enough that the feeding mechanism of *Psolus fabricii* can filter cells from the water column, contributing to gonadal production. An influence of food availability on gonad development is also suggested by numerous studies on other invertebrates (Sastry and Blake, 1971; Gimazane, 1972; Bayne, 1975). The vernal warming is another potential environmental change at this time, and our temperature record, which began in mid April in 1989, indicates that warming had occurred in April.

Role of nutritive phagocytes and the gonadal tubule wall in supporting gametogenesis

The appearance of nutritive phagocytes and their role in eliminating residual gametes is well documented in studies of holothurians (Tanaka, 1958; Costelloe, 1985; Smiley and Cloney, 1985) and other echinoderms (Liebman, 1950; Holland and Giese, 1965; Fenaux, 1972). In echinoids, nutritive phagocytes have been shown to transform reserves to dissolved compounds which are later used for gamete production (Holland and Giese, 1965). This may occur in *Psolus fabricii*, but since the phagocytes disappear before the active gametogenic period, these substances would have to be stored for their eventual use in gametogenesis.

In *Psolus fabricii*, the gonadal tubule wall thickens during the period of gametogenic inactivity, from autumn to mid winter (Fig. 10). This growth, coincident with the autumnal decrease in food availability, falling temperatures, and short photoperiod, appears to be a priority in the use of energetic reserves of the animal at this time. In a variety of echinoderms, gametogenesis is similarly preceded by a thickening of the tubule wall, and this is thought to represent an accumulation of reserves for gametogenesis (Pearse, 1969; Gonor, 1973). Costelloe (1985) suggests that certain increases in gonadal size in the sea cucumber *Aslia lefrevrei* are due to the storage of materials in the tubule wall, but *P. fabricii* does not show an increase in gonadal mass as the tubule wall thickened. The earlier growth of the tubular wall in the large tubules, compared with the small tubules, suggests that resources are channeled preferentially to the large tubules. This could be because the later stages of gametogenesis in the large tubules require more resources than the earlier stages in the small tubules (Fig. 10). In the small tubules, the massive proliferation of the earlier gametogenetic stages during January and February precedes the thinning of the wall that begins in March. This suggests that the proliferation does not require large amounts of reserves.

External spawning cues

A massive loss of gametes over a short period strongly suggests that spawning is controlled by external factors

(Himmelman, 1981; Giese and Kanatani, 1987; Starr, 1990; Starr *et al.*, 1990, 1992). In both years of our study, the gonadal indices, measurements of tubule diameter, and histological observations indicated an abrupt spawning between two successive sampling dates (a 4-weeks interval in 1988, and 2 weeks in 1989). Temperature more than any factor has been suggested as a spawning signal in invertebrates (Orton, 1914; Brown, 1984; Bricelij *et al.*, 1987), but we did not observe a consistent relationship between temperature and spawning in *Psolus fabricii*. Similar conclusions have been reported for other holothurians (Costelloe, 1985; Cameron and Fankboner, 1986). The only study suggesting that temperature might control spawning in holothurians is that of Tanaka (1958). That spawning time of *P. fabricii* varies between years suggests that spawning is not controlled by photoperiod.

Our data suggests that spawning in *Psolus fabricii* may be signaled by the phytoplankton increase. Therriault and Levasseur (1985, 1986) demonstrate that the phytoplankton bloom in the Estuary is always delayed relative to that of the Gulf of St. Lawrence because of freshwater run-off: the bloom only develops after the surface layer has stabilized, which occurs when the spring run-off drops. In both 1988 and 1989, spawning in *P. fabricii* coincided with the predicted onset of the bloom, based in turn on the decrease in freshwater run-off (Fig. 11). Such a synchrony is further indicated by the coincidence of the spawning dates of *P. fabricii* with those of the green sea urchin, whose spawning is triggered by phytoplankton (Himmelman, 1975; Starr *et al.*, 1990, 1992). Cameron and Fankboner (1986) indicated that phytoplankton may also initiate spawning in *Stichopus californicus*. They noted that spawning individuals in the field were almost only observed after periods of bright sunshine ($\geq$5 h d-1 for >4 d), and further that phytoplankton was abundant during some spawnings.

The spawning in 1989 resulted in the release of a larger amount of gametes than in 1988 (Fig. 4). Possibly more gametes attained maturity in 1989 because of the delay in spawning. We suggest this because the mean tubule diameter attained a higher value in 1989 than in 1988, and the difference was largely due to the growth that occurred during June and July (Fig. 4). The longer period before spawning may have permitted the completion of gametogenesis in additional tubules, tubules that might otherwise not have matured until the following year. In addition, the gonadal index and tubule diameter did not fall as low in 1988 as in 1989. This again suggests that fewer gametes were mature when the spawning cue was detected in 1988. This discontinuation of gamete production after the early 1988 spawning suggests that physiological mechanisms prevent further gamete maturation and secondary spawnings once spawning has occurred. For the urchin, for which phytoplankton has been shown to be the spawning cue, spawning in the laboratory increases with plankton abundance (Starr *et al.*, 1990). If this is true for *Psolus fabricii*, a more intense phytoplankton bloom in 1989 might account for the more massive spawning in that year. The greater mass of intestinal contents of *P. fabricii* in 1989 suggests there was a more intense bloom in that year (Fig. 11).

Planktonic stages of Psolus fabricii

Holothurians with small eggs usually have a larval stage, whereas species with large eggs usually develop directly into juveniles (Tanaka, 1958; Rutherford, 1973; Green, 1978; Tyler and Billett, 1988). *Psolus fabricii* has exceptionally large eggs, sometimes attaining 1400 μm in diameter, and lacks a larval phase (pers. obs.). Nevertheless, the juvenile stage is pelagic. Probably, as Tyler and Billett (1987) indicate for elasipodid holothurians, the abundant nutritive reserves in the egg account for the high degree of floatability of the pelagic stage. Warmer temperatures near the surface may enhance the rate of development, and in addition the pelagic juveniles may further benefit from increased food resources, either in the form of dissolved substances or planktonic cells. The feeding podia are well developed around the mouth of the pelagic juveniles of *P. fabricii* (pers. obs.), which suggests that they are capable of feeding on suspended particles.

Feeding

Some holothurians feed on organic material at the water-sediment interphase (Hyman, 1955; Reese, 1966; Ferguson, 1969) whereas others feed on planktonic particles (MacGinitie and MacGinitie, 1949; Brumbaugh, 1965). *Psolus fabricii* is a highly selective feeder. For example, although numerous phytoplankton and zooplankton species are common in the region where we collected *P. fabricii* (Côté, 1972; Cardinal and Lafleur, 1977; Fortier *et al.*, 1978; Maranda and Lacroix, 1983; Therriault and Levasseur, 1985, 1986), the intestines contained almost exclusively four species of diatoms. The proportion of these items decreases in abundance in the intestine as productivity drops in late autumn and winter and is replaced by nonliving matter. *P. chitonoides* (Fish, 1967) and *Cucumaria elongata* (Fankboner, 1978) similarly feed primarily on suspended living particles. That dendrochirotes are most abundant in temperate and subtropical waters, and rare in tropical areas and at great depths (Pawson, 1966; Hansen, 1975; Lawrence, 1987), suggests that they require the abundance of small living particles such as found in shallow water northern areas (Lawrence, 1987). Nonliving matter or detritus has been suggested to be an important source of food in the diet of suspension feeders (Baier, 1935; Newell, 1965; Kirby-Smith, 1976) and could provide nutritional resources for *P. fabricii*

during the winter. The long intestine of dendrochirotides may be an adaptation for digesting vegetal matter (Lawrence, 1987). *P. fabricii* has a remarkably long intestine relative to its body size (intestinal length = −1.68 + 4.52 dry body wall mass; r = 0.95, n = 37). For example, an adult measuring 6.1 cm in distance mouth-anus, (34 g) has a 152 cm intestine. This unusually long intestine may be an adaptation to its diet of diatoms which are protected by siliceous frustules.

Acknowledgments

We are greatly indebted to N. Piché for his help in collecting the samples and for the underwater photographs of *Psolus fabricii*. The aid of S. Paradis, M. Claereboudt, A. Duval, E. Bourget, A. Cantin, A. Cardinal, H. Guderley, L.-P. Hamel, O. Hamel, B. Laganière, and A. Tremblay at various points in the project is also gratefully acknowledged. Thanks are also due to A. Pusterla (Département de Pathologie, Université Laval) and A. J. Collet (Département d'Anatomie, Université Laval) and the Département d'Océanographie (Université du Québec à Rimouski) for the histological preparations. The first author was supported by a FCAR scholarship and the research was supported by NSERC funding to J. H. H. and L. D.

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